Free radical chemistry of Naphthol and its analogues: Experimental and theoretical studies

1-Naphthol and 2-naphthol (designated as **1** and **2**), isomeric hydroxyarenes, have been the subject of several experimental and associated theoretical studies. They are released into the environment through manufacture, handling, use and disposal in the context of their use in dye and pesticide industries. Further they enter into the environment owing to the oxidation of naphthalene (the major constituent of coal tar) or as metabolites of carbamate pesticides like sevin or carbaryl (1-naphthyl-N-methylcarbamate) by chemical and biological processes. The presence of hydroxyl group in naphthols leads to their increased solubility and portability in natural aquifers and are considered as more toxic than naphthalene and other polycyclic aromatic hydrocarbons. Both naphthols are used as biomarkers for livestock and humans exposed to polycyclic aromatic hydrocarbons.

Several comprehensive experimental/theoretical studies have been reported for the $^{\bullet}OH$ induced oxidation reactions of phenolic compounds in view of their so-called antioxidant or free-radical scavenging properties. In the case of phenol (C_6H_5OH), the $^{\bullet}OH$ reacts by addition to the aromatic ring that lead to the formation of the $^{\bullet}OH$-adduct as the preferred transient at neutral pH as demonstrated by ESR and pulse radiolysis studies. However, the $^{\bullet}OH$ addition essentially leads to the formation of isomeric adduct species. Acids and bases catalyze the dehydration of the primary $^{\bullet}OH$-adduct(s) to the formation of phenoxyl radical. Furthermore, the base catalyzed dehydration is reported to be faster than the acid catalyzed dehydration. Indeed, it was recognized that thermodynamically the formation of phenoxyl radical is more favorable than $^{\bullet}OH$ additions.

1,4-Naphthoquinone and their analogues have a variety of biological applications starting from anti-microbial to anti-cancerous. They are generally found in plants, fungi and in some animals. The anti-tumour activity of adriamycin and daunorubicin (structural analogues of naphthoquinone) is due to the semiquinone radicals produced from these quinone systems. Another important property of quinones is that they are very good electron acceptors. Lawsone (2-hydroxy naphthoquinone) commonly known as hennatonic acid is a red orange dye which is present in henna plant. Juglone (5-hydroxy naphthoquinone), another hydroxylated derivative of naphthoquinone is found in the root, leaves and bark of walnut trees. Lawsone and Juglone (Scheme 1) has been a subject of intensive experimental and theoretical studies and the biological activity of these naphthoquinones is found to increase in the presence of a substituted hydroxyl group. Because of their biological importance, a clear understanding of the free radical chemistry of such compounds is very necessary. They are known antioxidants and the pattern of radical reaction, *viz*., the reaction of hydroxyl radical ($^{\bullet}$OH), with hydroxy naphthoquinones such as lawsone and juglone are also investigated in the present study.

1-Naphthyl methyl carbamate (carbaryl) is a member of the carbamate insecticide class which is often used because of the instantaneous action. In India, it is commonly used against pests in agricultural fields and the pesticide consumption rate is next to benzene hexa chloride. It is used as a replacement for organochlorine insecticides. Carbaryl is a widely detected insecticide in surface waters and it claims the second position among the detected insecticides in surface water. Like all carbamates, carbaryl also inhibits the enzyme acetylcholine esterase at synapses. It can be somewhat toxic to mammals and is applying to more than 120 different varieties of crops and also as a garden insecticide.

The base hydrolysis of carbaryl can produce α-naphthol and the hydrolysis rate will be increased at high temperatures. The advanced oxidation processes (AOPs) for the

degradation of carbamate pesticides using reactive oxygen species is reported. $^{\bullet}OH$ is the main oxidant in majority of the AOPs like H_2O_2/ UV photolysis, Fenton, photo Fenton, sonolysis, TiO_2 photo catalysis etc. For studying the underlying mechanism and the kinetics of the reaction of $^{\bullet}OH$ with carbaryl, we employed pulse radiolysis which is a well known technique for detecting the transient intermediate of a radical reaction and its kinetics.

The capability of AOPs to achieve nearly complete mineralization is well documented in the case of a variety of pollutants. However, in certain cases, it is also probable that a number of intermediate products/ end-products which are even more toxic or equally toxic can be formed in the system, that are generally difficult to analyze because of very low concentration. Consequently, a detailed investigation of resulting transformation products are utmost important in the case of any degradation protocol. The analysis of the transformation products of the above mentioned compounds induced by H_2O_2/ UV photolysis was carried out using a high resolution mass spectrometer.

Organization of the thesis

The thesis comprises of five chapters; introduction and experimental methods cover the first two chapters. Chapter 3 presents the oxidation reactions of α and β-naphthols. This chapter describes the mechanism of primary oxidation reactions of isomeric naphthol molecules with $^{\bullet}OH$. The study using pulse radiolysis technique, allows the qualitative understanding of the preliminary oxidation products (the transient species) formed during the reaction of $^{\bullet}OH$ with α and β-naphthols. Rate constant determinations demonstrated the diffusion controlled nature of $^{\bullet}OH$ reactions of α and β- naphthols. Results of DFT calculations for $^{\bullet}OH$ reactions of both naphthols provide a conceptual framework of the most applicable reaction mechanism. The products of the reaction of α and β- naphthols under H_2O_2/ UV photolysis have also been explained in this chapter.

Chapter 4 describes the $^{\bullet}OH$ induced oxidation reactions of 2- and 5- hydroxy naphthoquinones (lawsone and juglone). The transient intermediate and the products of the reaction of $^{\bullet}OH$ with lawsone and juglone were described in this chapter. A detailed product analysis was carried out using LC-Q-TOF-MS analysis. The dimerisation and disproportionation reactions of lawsone and juglone towards $^{\bullet}OH$ are explained in this chapter.

The free radical chemistry of carbaryl (naphthyl methyl carbamate) is presented in chapter 5. The transient intermediates of the reaction of $^{\bullet}OH$ and $SO_4^{\bullet-}$ with carbaryl is described using pulse radiolysis experiments. Theoretical calculations to support the experimental findings were also presented in this chapter. A detailed product analysis of the reaction of $^{\bullet}OH$ with carbaryl is explained using LC-Q-TOF-MS analysis.

Chapter 1

Introduction and Objectives

This chapter discusses the principles involved in various modes of interaction of radiation with matter. Different types of radiation sources, the radiation chemistry of water, the properties of radicals derived from water radiolysis and their explicit interaction with matter in the context of radiation chemical oxidation have been discussed. The elementary quantum mechanics and the basics of density functional theory method employed in the thesis are also presented. The chapter contains an overview and background of the thesis too.

Chapter 2

Materials and methods

The details of the materials used and the experimental and theoretical methods adopted for the thesis has been presented in this chapter. The pulse radiolysis set up and techniques for selective generation of radicals in the experimental studies are discussed. A brief description of some generic terms used in the theoretical calculation sections of the thesis has also been included. The $^{\bullet}OH$ generation methods are also discussed especially H_2O_2/UV photolysis. The techniques like HPLC and LC-Q-TOF/MS are explained in this chapter.

Chapter 3

Oxidation reactions of 1- and 2-naphthols: experimental and theoretical study

In this chapter the transients formed during the reactions of oxidizing radicals with 1-naphthol (**1**) and 2-naphthol (**2**) in aqueous medium has been investigated by pulse radiolysis with detection by absorption spectroscopy and DFT calculations. The transient spectra formed on hydroxyl radical ($^{\bullet}OH$) reactions of **1** and **2** exhibited λ_{max} at 340 nm and 350 nm at neutral pH. The rate constant of the $^{\bullet}OH$ reactions of **1** (**2**) were determined from build-up kinetics at λ_{max} of the transients as 9.6×10^9 $M^{-1}s^{-1}$ (7.3×10^9 $M^{-1}s^{-1}$). DFT calculations using B3LYP/6-31+G (d,p) method have been performed to locate favorable reaction sites in both **1** and **2**. Calculations demonstrated that $^{\bullet}OH$ additions can occur mostly at C1 and C4-positions of **1,** and at C1 and C8-positions of **2**. Among several isomeric $^{\bullet}OH$ adducts possible, the C1 adduct, was found to be energetically most stable both in **1** and **2**. TDDFT calculations in solution phase have shown that experimental spectrum of **1** was mainly attributed by $\mathbf{1a_4}$ (kinetically driven $^{\bullet}OH$-adduct) formed via the addition of $^{\bullet}OH$ at C4-position which was 0.73 kcal/mol endergonic than $\mathbf{1a_1}$ (thermodynamic $^{\bullet}OH$-adduct); whereas $\mathbf{2a_1}$ (thermodynamic/kinetic $^{\bullet}OH$-

adduct) was mainly responsible for the experimental spectrum of **2**. Naphthoxyl radicals of **1** and **2** have been predicted as the transient formed in the reaction of $^{\bullet}OH$ at basic pH. In addition, the same transient species resulted from the reactions of oxide radical ion ($O^{\bullet-}$) at pH~13 and azide radical ($N_3^{\bullet}$) at pH 7 with **1** and **2**. Further, UV photolysis of aqueous solutions of **1** and **2** containing H_2O_2 (UV/H_2O_2) were used for the $^{\bullet}OH$ induced oxidation product formations up on 60% degradations of **1** and **2**; profiling of the oxidation products were performed by using an ultra-performance liquid chromatography quadrupole time of flight mass spectrometry (UPLC–Q-TOF) method. According to the UPLC–Q-TOF analyses, the preliminary oxidation products are limited to di-hydroxy naphthalenes and naphthoquinones with N_2-saturation, while some more additional products (mainly isomeric mono- hydroxy naphthoquinones) have been observed in the degradations of **1** and **2** in presence of O_2. We postulate that, di-hydroxy naphthalenes are derived explicitly from the most favorable $^{\bullet}OH$-adducts speculated (preference is in terms of kinetic/thermodynamic dominancy of transients) by using theoretical calculations which in turn substantiate the proposed reaction mechanism. The observation of $^{\bullet}OH$-adducts for an aromatic phenol (herein for both **1** and **2** at pH 7) rather than phenoxyl type radical in the pulse radiolysis experiments is a distinct and unique illustration. The present study provides a meaningful basis for the early stages associated with the $^{\bullet}OH$ initiated advanced oxidation processes of 1- and 2-naphthols. The transient absorption spectra observed during the reaction of $^{\bullet}OH$ with 1 and 2 at two different pH is shown in figure 1 and the optimized geometries of (**a**) 1-naphthol and (**b**) 2-naphthol with selected bond lengths in Å unit. HOMO of (**c**) 1-naphthol and (**d**) 2-naphthol are color-mapped onto van der Waal's surfaces and the MO coefficients are given in figure 2 and the possible route of product formations in the oxidation of 1-naphthol by UV/H_2O_2 method with N_2–saturation is given in scheme 1.

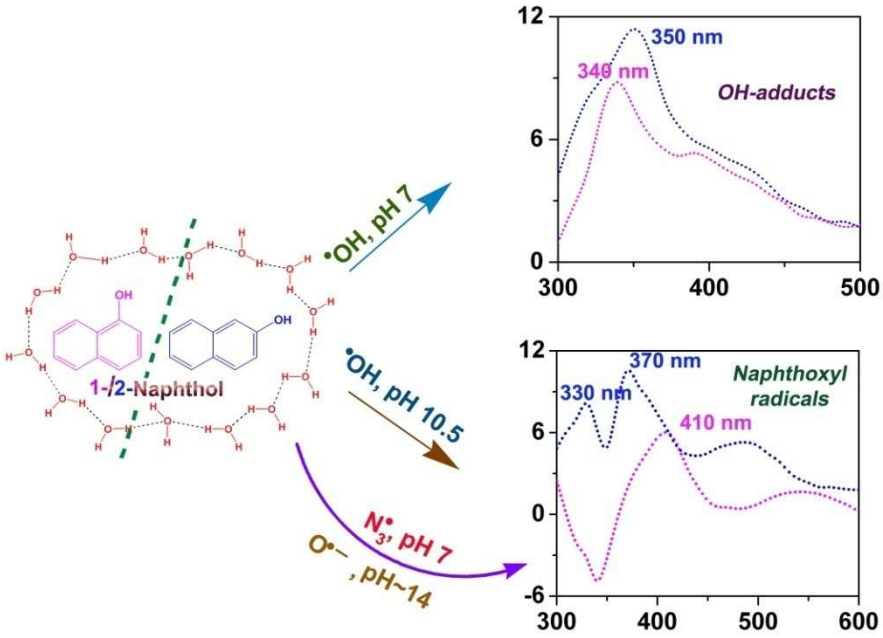


Figure 1. A schematic representation of the transient intermediates observed during the reaction of •OH with 1- and 2-naphthols

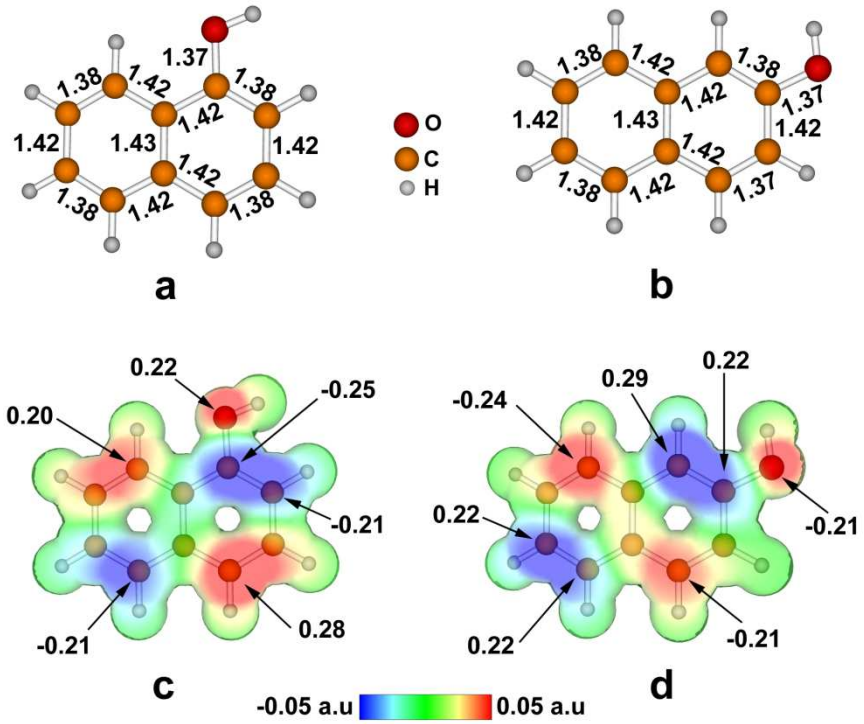
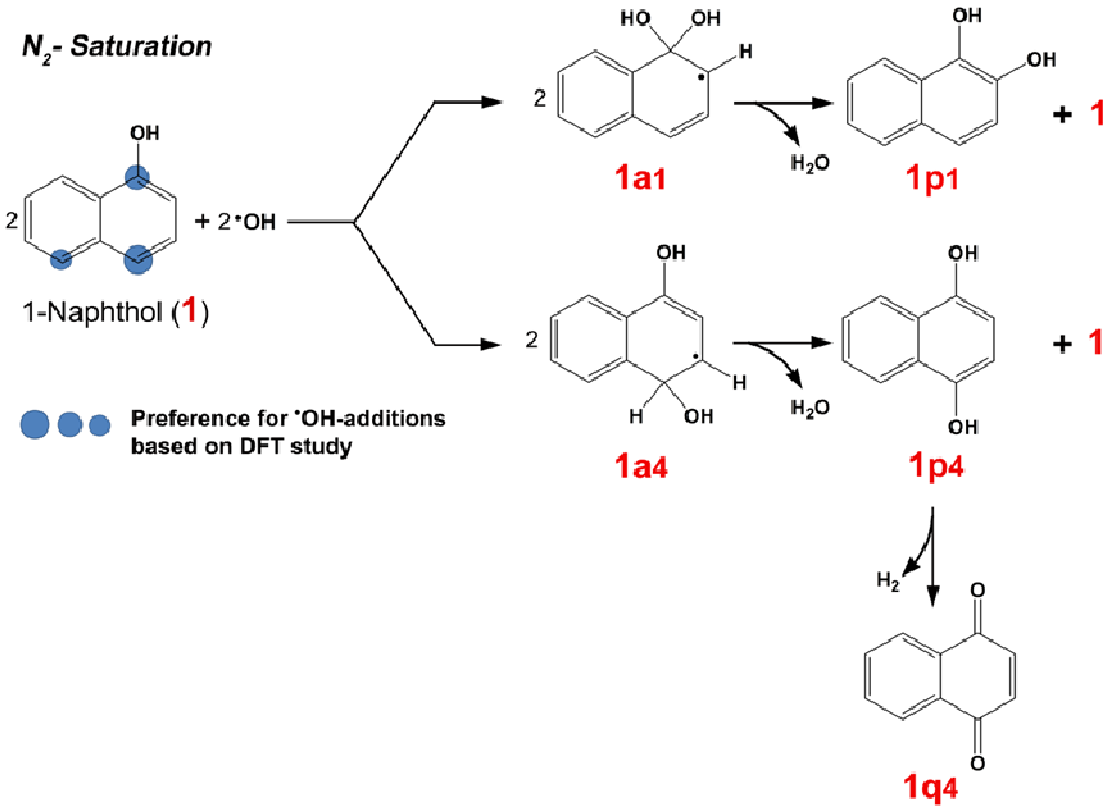


Figure 2. Optimized geometries of (**a**) 1-naphthol and (**b**) 2-naphthol with selected bond lengths in Å unit. HOMO of (**c**) 1-naphthol and (**d**) 2- naphthol



Scheme 1. A possible route of product formations in the oxidation of 1-naphthol by UV/H_2O_2 method with N_2–saturation

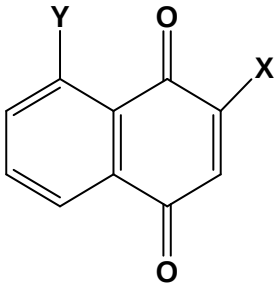
Chapter 4

Oxidation reactions of hydroxy naphthoquinones: Mechanistic investigation by LC-Q-TOF-MS Analysis

The radical chemistry of naphthoquinones and their analogues are important as they are known for their anti-tumour activities. The hydroxyl radical ($^{\bullet}$OH) induced oxidation reactions of isomeric hydroxy naphthoquinones namely, lawsone and juglone, were carried out. The initially formed transient intermediates were monitored and a detailed product study was carried out. H_2O_2/ UV photolysis experiments of both lawsone and juglone was carried out using a medium pressure high intensity (254 nm) mercury lamp. The degradation products were analysed using a high resolution mass spectrometer (LC-Q-TOF-MS). The transient intermediates were detected using a picosecond electron accelerator (ELYSE).

Hydroxylated adducts of both lawsone and Juglone were detected during the product analysis with the reaction of $^{\bullet}$OH. Isomeric mono-hydroxylated adducts (m/z 191.0346 (ESI+) and 189.0186 (ESI-) were detected at four different RTs for lawsone and at five different RTs for juglone. The isomeric mono- hydroxylated adducts of lawsone were confirmed using survival yield (SY) analysis. The isomeric mono-hydroxylated products undergo further $^{\bullet}$OH addition resulting in di-hydroxylated derivatives. The hydroxylated adducts of lawsone undergoes dimerisation resulting in m/z 347.0545 (dimerisation of two mono hydroxylated adduct radicals) and m/z 363.0500 (dimerisation of a mono-hydroxylated adduct and a di-hydroxylated adduct radical of lawsone). However no such dimerisation was detected in the case of juglone. The transient spectral analysis using pulse radiolysis studies revealed the formation of hydroxycyclohexadienyl type radical of both lawsone and juglone as the initially formed intermediate.

The $^{\bullet}$OH induced reactions of both lawsone and juglone results in the mono and di-hydoxylated derivatives. However dimerisation of the hydroxylated adducts radicals was observed only in the case of lawsone. With the transient analysis and the product studies a detailed reaction mechanism has been proposed. The demonstration of the various isomeric products using mass spectrometry is a clear proof of the addition probability of $^{\bullet}$OH at different positions of lawsone and juglone, which is generally a difficult task using other analytical techniques. Knowledge of the $^{\bullet}$OH induced oxidation of these types of biologically relevant system has utmost importance in radiation biology. Structure of 2 and 5-hydroxy naphthoquinones are given in scheme 2.



X=OH, Y=H, Lawsone

X=H, Y=OH, Juglone

Scheme 2. Structure of 2 and 5- hydroxy naphthoquinones

The transient absorption spectra recorded during the reaction of $^{\bullet}$OH with 2 and 5-hydroxy naphthoquinones is shown in figure 3 and a detailed reaction mechanism is given in scheme 3

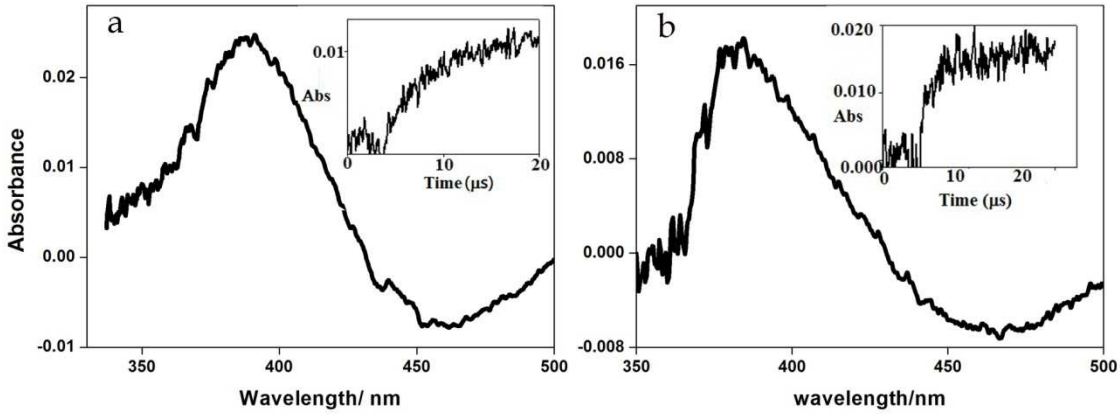
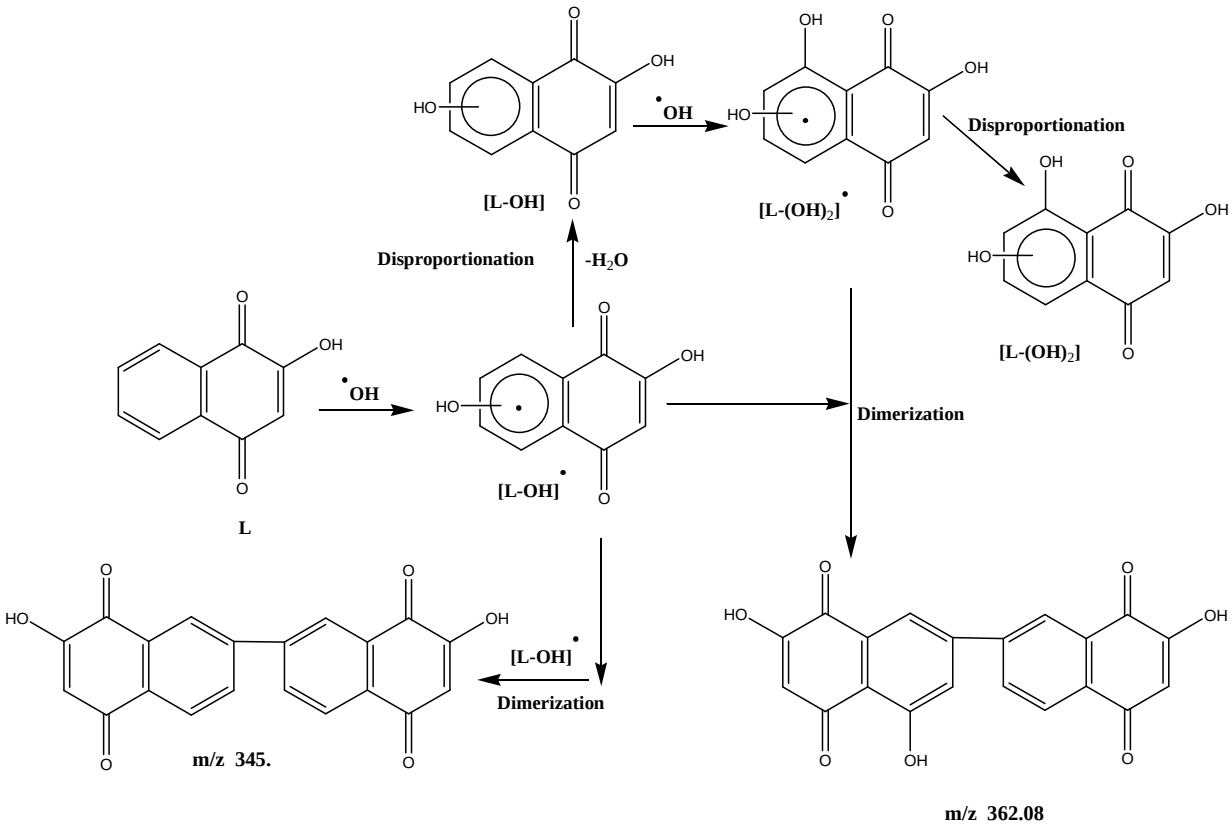


Figure 3. Transient absorption spectra recorded during the reaction of $^{\bullet}$OH with juglone (a) and lawsone (b). Inset: (a) Absorption trace recorded during the reaction of $^{\bullet}$OH with juglone at 390 nm, 20 μs and inset: (b) absorption trace recorded during the reaction of $^{\bullet}$OH with lawsone at 380 nm, 25 μs



Scheme 3. Proposed pathway of the disproportionation and dimerisation reactions of lawsone on reaction with $^{\bullet}OH$

Chapter 5

Oxidation reactions of carbaryl in aqueous solutions

In this chapter, the free radical ($^{\bullet}OH$ and $SO_4^{\bullet-}$) induced oxidation reactions of carbaryl are explained based on pulse radiolysis, product analysis and theoretical calculations. The transient intermediate during the reactions of $^{\bullet}OH$ and $SO_4^{\bullet-}$ with carbaryl was monitored using pulse radiolysis. The hydroxyl adduct of carbaryl was detected as the transient intermediate and the calculated value of the bimolecular rate is found to be diffusion controlled. On the other hand the reaction of $SO_4^{\bullet-}$ with carbaryl results in one electron oxidation. The radical cation was detected as the transient intermediate in this case. The transient absorption spectra recorded during the above two reactions are given in figure 4 and 5.

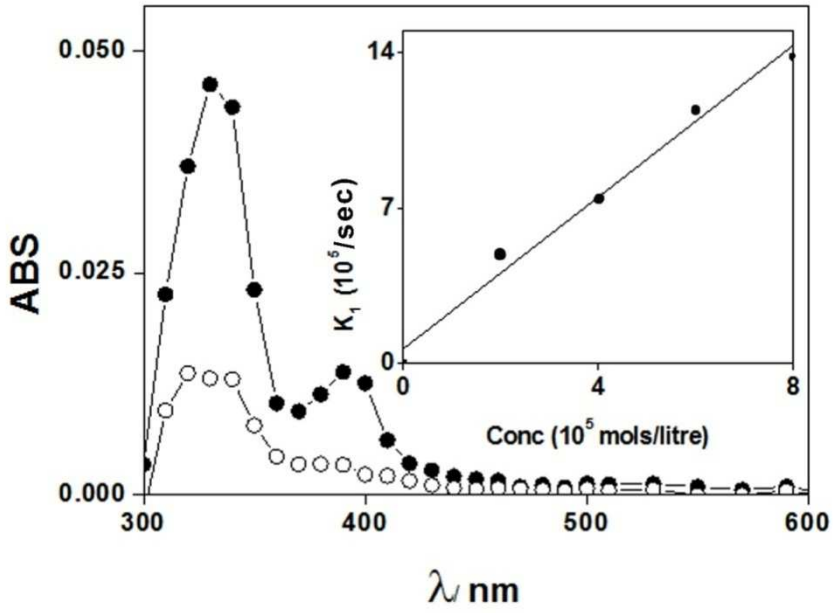


Figure 4. Transient absorption spectrum recorded (●) 10 µs and (○) 80 µs after the pulse during the reaction of $^{\bullet}OH$ with carbaryl. Inset: Plot of pseudo first order build-up against concentration for the reaction of $^{\bullet}OH$ with carbaryl at 330 nm

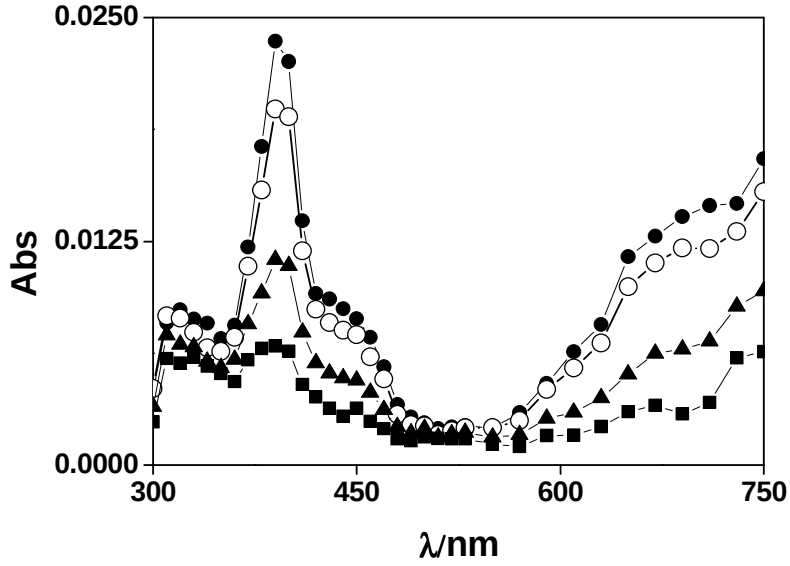
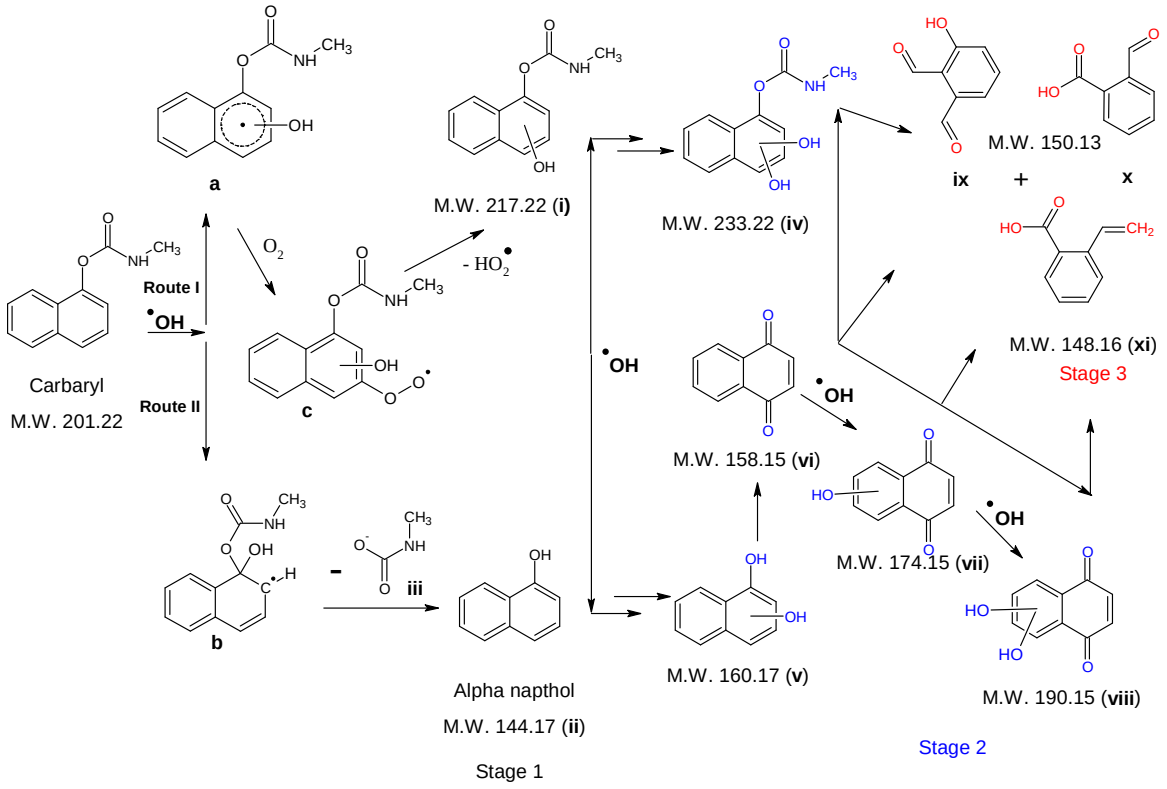


Figure 5. Transient absorption spectrum recorded during the reaction of $SO_4^{\bullet-}$ with carbaryl. (●) 2.8 µs, (○) 10 µs, (▲) 40 µs and (■) 90 µs windows respectively

All the computations were performed using Gaussian 09 suite of programs. Geometry optimizations and harmonic vibrational frequency analysis both in gas and solution phases (using polarizable continuum model– PCM were performed with the M05 method using the

6-311++G** basis set. M05 is reported to be one of the best method in explaining chemistry associated with radicals. Reaction of •OH with carbaryl at pH 7 was carried out using UV irradiation in the presence of H_2O_2 (UV/H_2O_2), which provides a continuous supply of •OH. Photo-irradiations were carried out using a medium pressure high intensity (254 nm) mercury lamp. Products were analyzed using a Waters ACQUITY UPLC H-Class system coupled with a Waters Xevo G2 quadrupole-time-of-flight (Q-TOF) high resolution mass spectrometer which uses electrospray ionization (ESI) technique. The proposed pathway of carbaryl on its reaction with •OH is shown in scheme 4. The proposal is based on the nature of short lived intermediate products detected by pulse radiolysis technique as well as the stable transformation products identified by high resolution mass spectrometric analysis. The exact reaction sites located by theoretical calculations were also utilized here. On the basis of the results, the degradation pathways of carbaryl are categorized into three stages (stage 1-3) described in scheme 4.



Scheme 4. Proposed mechanism of the reaction of carbaryl with •OH

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2. **R. Sreekanth**.; M. M Sunil Pau1.; U. K Aravind.; J. L. Marignier.; J. Belloni.; C.T Aravindakumar; Oxidation reactions of hydroxy naphthoquinones: Mechanistic investigation by LC-Q-TOF-MS Analysis. ***Int. J. Radiat Biol.*** **2014** (in press)

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Sreekanth R., Sunil Paul M. M., Aravind U. K., Aravindakumar C. T.; *Hydroxyl Radical Mediated Oxidation of 1- and 2-Naphthols: A Mass Spectrometric Study, Proceedings of the International Conference on Frontiers of Mass Spectrometry (ICMS 2013)(September 6-9, 2013), Kottayam*

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Sreekanth R., Sunil Paul M. M., Aravind U. K., Marignier J. L., Belloni J., Aravindakumar, C. T.; *Identification of the Transformation Products of Hydroxy Naphthoquinones: A Mass Spectrometric Study*, *Proceedings of the International Conference on Frontiers of Mass Spectrometry (ICMS 2013)(September 6-9, 2013), Kottayam*

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